Irradiation Temperature Influences Product Quality Factors of Frozen Vegetables and Radiation Sensitivity of Inoculated *Listeria monocytogenes*†

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ABSTRACT

Four frozen vegetables (broccoli, corn, lima beans, and peas) were gamma irradiated at subfreezing temperatures ranging from −5 to −20°C to determine (i) the radiation sensitivity of an inoculated outbreak strain of *Listeria monocytogenes* (ATCC 49594), (ii) the effect of changing irradiation conditions (i.e., temperature) on that sensitivity, and (iii) the effect of the recommended radiation dose on the texture and color of irradiated frozen vegetables. The amounts of radiation necessary to reduce the bacterial population by 90% (D_{10}-values) for *L. monocytogenes* differed significantly among vegetables at each irradiation temperature. D_{10} increased significantly with decreasing temperature for all vegetables, with each vegetable showing a different response pattern. At an irradiation temperature of −5°C, D_{10} ranged from 0.505 kGy for broccoli to 0.613 kGy for corn. At −20°C, D_{10} ranged from 0.767 kGy for lima beans to 0.916 kGy for peas. At −20°C, radiation doses sufficient to achieve a 5-$\log_{10}$ kill (3.9 to 4.6 kGy) caused significant softening of peas and broccoli stems but not of corn or lima beans. Lower doses of comparable antimicrobial efficacy delivered at −5°C (2.5 to 3.1 kGy) did not cause significant changes in texture in any vegetable. Color varied significantly among the dose-temperature combinations only for broccoli florets; this variation did not demonstrate a clear pattern of quality changes in response to irradiation.

Listeria monocytogenes is a foodborne bacterium that has been responsible for numerous foodborne illness outbreaks and product recalls *(1)*. *L. monocytogenes* can contaminate a variety of food products, including vegetables *(2, 3)*. The possibility of contamination of food products increases with additional handling and processing steps *(4)*. Although the practice of eliminating the terminal point-of-consumption blanch step is not recommended by processors of frozen vegetables, it is used by food preparers to achieve a more desirable taste and texture in vegetables such as peas *(9)*. *L. monocytogenes* is capable of growth at refrigeration temperatures *(10)*; frozen vegetables that are thawed but not blanched for use in salsas and salads, even those held chilled or refrigerated, may therefore pose a risk.

Ionizing radiation has been shown to effectively eliminate *L. monocytogenes* on processed meat products; the radiation sensitivity of the bacteria is influenced by the composition of the food substrate *(13)*. The primary mode of action of ionizing radiation is the creation of hydrogen and hydroxyl radicals from water molecules *(5)*. Under conditions of limited free water, such as in frozen products, higher radiation doses are typically required to reduce the bacterial population, with radiation resistance typically increasing to a maximum near −20°C *(12, 14)*. The effective radiation dose for the elimination of *L. monocytogenes* has been researched for a variety of fresh and frozen products of animal origin *(7)*.

When conducted at refrigeration temperatures, irradiation can lead to softening of fresh vegetable products due to hydrolysis of pectins *(16)* and can induce changes in color and vitamin content *(6)*. The microbiology and sensory properties of irradiated fresh fruits and vegetables are areas of active research *(8)*; the irradiation of frozen vegetables has been less well explored *(7, 15)*. The objectives of this study were to determine (i) the effect of decreasing temperature on the radiation sensitivity of an outbreak strain of *L. monocytogenes* at frozen temperatures on a vegetable substrate, (ii) the effect of changing vegetable substrates on that sensitivity, and (iii) the effect of the recommended radiation dose on the texture and color of irradiated frozen vegetables.

MATERIALS AND METHODS

Pathogen. Stock cultures of the Scott A strain of *L. monocytogenes* (ATCC 49594; American Type Culture Collection, Manassas, Va.) were maintained on 50% glycerol at −70°C. A frozen culture was regrown in tryptic soy broth (Difco Laboratories, Detroit, Mich.) for 16 h at 37°C with agitation, streaked onto Palcam agar (Difco), and incubated at 37°C for 48 h to form single colonies. These colonies were used to inoculate fresh tryptic soy broth for each experiment and grown for 16 h at 37°C with agitation. The cell density of the starting inoculum was determined by serial dilution with sterile Butterfield's phosphate buffer (BPB; Applied Research Institute, Newtown, Conn.) and pour plating with tryptic soy agar (Difco). The cell density was typically 10^9

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† Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

CFU/ ml. Aliquots (200 ml) of starting inoculum were mixed with 1,800 ml of sterile BPB to produce the working inoculum.

Vegetables. Frozen vegetables were purchased in bulk from local markets and stored at −30°C until they were used. In order to test surface anatomical features for influence on radiation sensitivity, four vegetables were chosen on the basis of their distinct surface features: green peas (*Pisum sativum*) and lima beans (*Phaseolus lunatus*) (waxy and smooth surfaced), sweet corn (*Zea mays*) (waxy with a partially roughened surface), and broccoli (*Brassica oleracea* var. *italica*) florets (nonwaxy with a complex, involuted surface). Before inoculation, the commercial packaging was opened aseptically, and the frozen vegetables were transferred to a sterile plastic bin and allowed to thaw at room temperature. The products were determined to be free of background microflora (<10 CFU/g) by surface washing and serial dilution with BPB, pour plating with tryptic soy agar, and incubation of the plates at 37°C overnight.

Inoculation. Each vegetable was inoculated separately. Thawed vegetables (900 g) were transferred to a sterile glass inoculation dish (22 by 33 by 5 cm) in a biological airflow hood, and 1,000 ml of the working inoculum was added to the dish. The material was agitated gently for 120 s and then transferred to a sterile salad spinner–type centrifuge (Oxo International, New York, N.Y.). The material was spun twice to remove excess inoculum from the surfaces of the vegetables. Samples (45 g) of each vegetable were placed in no. 400 stomacher bags (Tekmar, Inc., Cincinnati, Ohio), which were then heat sealed. Immediately after heat sealing, the bags were covered in dry ice to rapidly freeze the samples.

Irradiation. Samples were held on dry ice prior to tempering to the intended irradiation temperature, typically for 30 to 60 min. The samples were tempered in a refrigerated bath (Cole-Parmer, Vernon Hills, Ill.) filled with a 50:50 mix of ethylene glycol and water. The samples were completely submerged for at least 15 min in the pre-set refrigerated bath before irradiation. This interval was selected on the basis of preliminary experiments with this equipment to ensure complete tempering of the samples. The temperatures of the samples were set to −5, −10, −15, and −20°C (±1°C). Immediately prior to irradiation, the samples were removed from the bath, wiped, and placed in a polypropylene bucket inside the irradiator chamber prechilled to the appropriate subfreezing temperature. Temperature control was maintained during irradiation by the injection of gas-phase liquid nitrogen into the sample chamber.

The inoculated samples were treated with 0.0 (control), 0.5, 1.0, 1.5, 2.0, and 3.0 kGy. For each vegetable-temperature combination, the experiment was performed three times in separate trials using separately prepared sets of vegetables samples. These samples were irradiated concurrently. All irradiation runs on a given day were conducted at a single temperature to maintain consistency in temperature control. The samples were irradiated at 0.098 kGy/min with a Lockheed-Georgia (Manetta, Ga.) cesium-137 self-contained gamma radiation source. Alanine pellets (Bruker, Inc., Billarna, Mass.) were used for dosimetry. The pellets were read on a Bruker EMS 104 EPR analyzer and compared with a previously determined standard curve. The actual dose was typically within 5% of the nominal dose.

Sampling. After irradiation, the samples were returned to dry ice until microbiological sampling was carried out, typically for 30 to 60 min. The bagged samples were thawed at room temperature and aseptically opened. Sterile BPB (180 ml) was added to the stomacher bag and agitated for 60 s. A 1-ml sample was withdrawn for serial dilution with sterile BPB. Pour plating with tryptic soy agar was used to determine the surviving bacterial population. Three pour plates per dilution were incubated for 48 h at 37°C and counted with an automatic plate counter. The data for each isolate were normalized against the control and plotted as the $\log_{10}$ reduction for the nominal doses. The slopes of the individual survivor curves were calculated by linear regression with a computer graphics program (SigmaPlot 5.0, SPSS Inc., Chicago, Ill.). The D_{10}-value (the radiation dose necessary to inactivate 90% of the population) was calculated by taking the negative reciprocal of the survivor curve slope (QuattroPro, Corel Corp., Ottawa, Ontario, Canada).

Color and texture. To determine the effect of ionizing radiation on the color and texture of the frozen vegetables, noninoculated samples (45 g) of each of the four vegetables were bagged and frozen in dry ice as described. The bagged samples were tempered to either −5 or −20°C for at least 15 min. A set of samples was tempered to −5°C and maintained at −5°C throughout irradiation. These samples were treated with the range of doses sufficient to achieve a 5-log (99.999%) kill based on previously determined D_{10}-values at −5°C: 0.0 (control), 2.5, and 3.1 kGy. A separate set of samples was similarly tempered to and irradiated at −20°C. These samples were treated with a range of higher doses necessary to achieve the same 5-log kill level based on the previously determined D_{10}-values at −20°C: 0.0 (control), 3.9, and 4.6 kGy. Dosimetry and temperature control were as described above. After irradiation, the bagged samples were stored for 2 to 6 days at −30°C until analysis was carried out. The samples were thawed at room temperature and opened immediately prior to analysis.

Color values were obtained with a Hunter Lab Miniscan XE meter (Hunter Laboratory, Inc., Reston, Va.) to determine the brightness (L value), greenness-redness (a value), and blueness-yellowness (b value) of the material. Broccoli florets were arranged with the florets completely covering the lens surface; peas, corn, and lima beans were arranged in closely spaced groups. The meter was calibrated with white and black standard tiles. Illuminate A, 10° standard observer, and a 2.5-cm port/viewing area were used. Four samples were analyzed for each temperature-dose combination. The experiment was performed three times in separate trials using separately prepared sets of vegetable samples.

Maximum shear force values were obtained with a TA-XT/2i5 texture analyzer with the TextureExpert 4.0 software package (Texture Technologies, Robbinsville, N.J.). The probe was a TA-7 Warner-Bratzler blade. The probe height calibration was set to a 10-mm return above the sample height, with a total probe travel distance of 23 mm. Eight samples were taken for each parameter. Each sample consisted of a single corn kernel, pea, bean, or transverse section of broccoli stem. The orientation of the corn and bean samples was with the distal-proximal axis over the blade slot so as to bisect the kernel or bean. Peas were oriented to bisect the sphere. Broccoli stems were sliced transversely just proximal to the first branch point, and two adjacent 5-mm-thick stem sections were taken from each of the four florets. The roughly circular samples were laid flat and bisected by the blade. The experiment was performed three times in separate trials.

Statistical analysis. The sensory characteristic data were evaluated by analysis of variance (SigmaStat, version 4.0, SPSS) using data pooled from the three trials, with final population sizes per dose-temperature combination of $n = 12$ for color intensity and $n = 24$ for shear strength. To determine the response of *L. monocytogenes* to irradiation, the significance of differences between the slopes for (i) each vegetable at the four temperatures

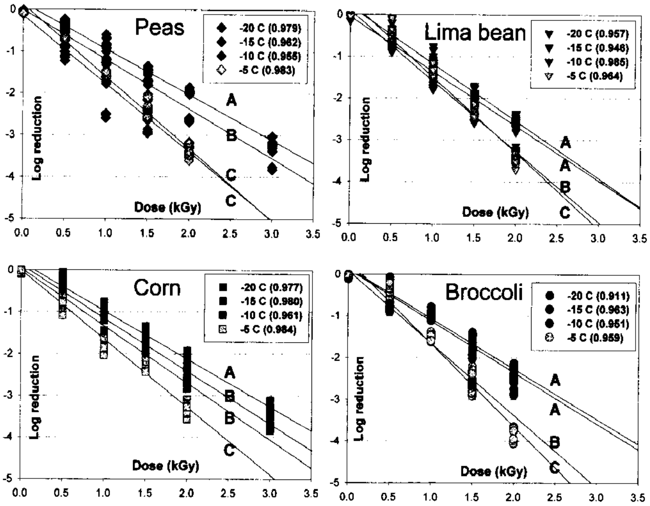

FIGURE 1. *Reduction of populations of* L. monocytogenes *ATCC 49594 on irradiated frozen vegetables. Letters within each graph correspond to (from top to bottom) −20, −15, −10, and −5°C. Symbols in the legend are followed in parentheses by the* r^2 *values of the linear regression. Regression lines with different letters are significantly different from each other* ($P < 0.05$) *as determined by analysis of covariance.*

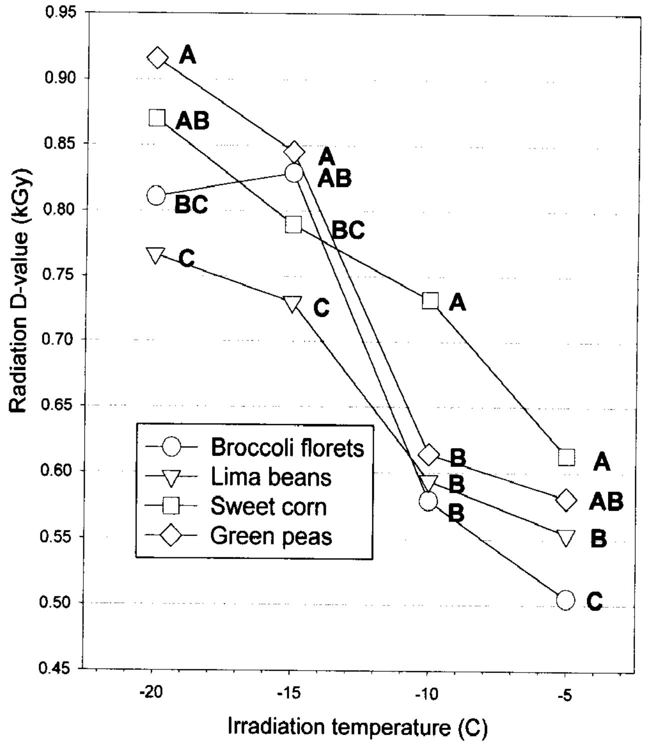

FIGURE 2. D_{10}*-values for* Listeria monocytogenes *ATCC 49594 on irradiated frozen vegetables. Within each temperature,* D_{10}*-values with different letters are significantly different* ($P < 0.05$) *as determined by analysis of covariance.*

and (ii) the four vegetables at each temperature was determined by analysis of covariance (Excel, Microsoft Corp., Redmond, Wash.) using data pooled from the three trials, with a final population size of $n = 9$ per dose-temperature combination. A four-parameter Gompertz model was used to describe the relationship of D_{10} versus irradiation temperature for each vegetable (SigmaPlot 5.0, SPSS) on the basis of D_{10} data from the entire study. The model is a sigmoidal rise to maximum from an initial value and takes the form $y = y_0 + ae^{e[-(x-x_0/b)]}$, where x is temperature (°C), y is D_{10} (kGy), and a, b, x_0, and y_0 are constants.

RESULTS

Ionizing radiation reduced the viable population of *L. monocytogenes* on the inoculated vegetables at every temperature evaluated (Fig. 1). The populations remaining on broccoli and lima beans that were subjected to the highest dose delivered (3.0 kGy) were not detectable for any temperature. At each temperature examined, the sensitivity of *L. monocytogenes* differed significantly among the four vegetables. The maxima exceeded the minima by 21, 26, 16, and 19% at −5, −10, −15, and −20°C, respectively. The products for which the maxima and minima were obtained varied from temperature to temperature.

The sensitivity of *L. monocytogenes* to ionizing radiation decreased with decreasing temperature for all vegetables (Fig. 2). At the extreme temperatures examined (−5 to −20°C), the D_{10}-values increased by 38, 42, 58, and 61% for lima beans, corn, peas, and broccoli, respectively (Fig. 2). A four-parameter Gompertz model (sigmoidal rise to maximum) adequately described the pattern of increasing D_{10} with decreasing temperature (Table 1). The coefficients for this model varied among vegetables, demonstrating a variable nonlinear relationship between D_{10} and temperature depending on the substrate.

At −20°C, radiation doses sufficient to achieve a 5-log kill caused significant softening in peas and broccoli stems but not in corn or lima beans (Fig. 3). At −5°C, lower doses with comparable antimicrobial efficacy did not cause significant softening in any of the vegetables examined. Significant variation was observed among all of the dose-temperature treatments for lima beans, but this variation was not indicative of a treatment response.

The color properties of corn and peas did not vary significantly among the dose-temperature combinations (Table 2). Significant variation was observed among the dose-temperature treatments for lima beans with respect to greenness-redness and for broccoli with respect to all of the parameters measured (Table 2). This variation was not indicative of a consistent treatment response. Where significant variation existed, the difference between maxima and minima was typically 15%, as determined quantitatively; these differences were not readily apparent to the naked eye.

TABLE 1. *Parameters of a Gompertz model*[a] *of the relationship of* D_{10} *to irradiation temperature in the subfreezing temperature range*

	a	b	x_0	y_0	r^2
Peas	0.3455	−2.308	−11.97	0.581	0.99
Broccoli florets	0.3150	−0.357	−10.13	0.505	0.99
Corn	2.3018	−16.522	20.82	−1.250	0.99
Lima beans	0.2191	−2.440	−11.30	0.554	0.99

[a] The four-parameter Gompertz equation describes a sigmoidal rise to maximum from an initial value and takes the form $y = y_0 + ae^{e[-(x-x_0/b)]}$, where x is temperature (°C), y is D_{10} (kGy), and a, b, x_0, and y_0 are constants.

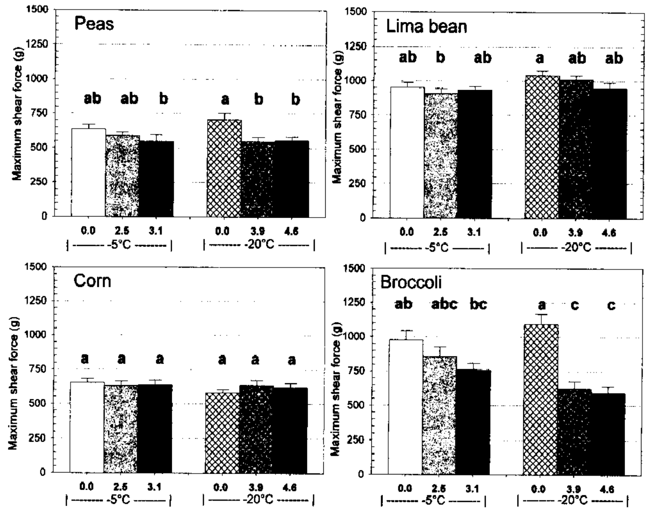

FIGURE 3. *Texture (maximum shear force) of irradiated frozen vegetables. The irradiation dose (kGy) is listed at the base of each bar, with bars grouped by temperature of irradiation (either −5 or −20°C). Within each graph, treatments with different letters are significantly different (*P *< 0.05) as determined by analysis of variance. Bars indicate standard error.*

DISCUSSION

In this study, the sensitivity of *L. monocytogenes* ATCC 49594 to ionizing radiation was found to be influenced by temperature and vegetable substrate. In combination, these two factors increase the complexity of observed behavior, as the response of *L. monocytogenes* to decreasing temperature on any of the vegetables tested was not predictably similar to that on any other. Anatomical similarity of the vegetables did not predict a similarity of influences on the inoculated bacteria. Peas and lima beans are anatomically similar with regard to surface characteristics, yet from −10 to −15°C, the D_{10}-value for *L. monocytogenes* increased by 38% on peas versus 22% on lima beans. Over the same range, the D_{10}-value increased by 43% on broccoli and by just 8% on corn. Comparable differences in response are evident among all of the vegetable-temperature combinations examined, demonstrating the complexity of the interactions. The Gompertz model employed to describe the relationship of temperature with D_{10} for each vegetable adequately describes the data obtained in these studies. It should be noted, however, that a more appropriate model, based on the mechanisms governing the effects observed, requires a clearer understanding of these mechanisms. These mechanisms may relate to surface chemistries, which may possibly involve anatomical microniches where inoculated bacteria could accumulate. The

TABLE 2. *Brightness and color of irradiated frozen vegetables*

Product	Temperature (°C)	Dose (kGy)	L (brightness)[a]	a (green/red)[b]	b (blue/yellow)[c]
Broccoli	−5	0.0	25.66 AB[d]	−8.31 AB	12.90 B
	−5	2.5	29.16 AB	−8.70 A	14.46 AB
	−5	3.1	26.33 AB	−8.56 A	13.12 AB
	−20	0.0	27.07 B	−7.93 AB	12.52 AB
	−20	3.9	26.29 A	−7.44 B	12.81 A
	−20	4.6	26.41 AB	−7.51 B	12.85 AB
Corn	−5	0.0	54.54 A	10.09 A	30.09 A
	−5	2.5	54.38 A	9.71 A	29.66 A
	−5	3.1	54.77 A	9.67 A	29.55 A
	−20	0.0	54.93 A	10.23 A	30.07 A
	−20	3.9	54.99 A	9.68 A	29.78 A
	−20	4.6	54.66 A	9.61 A	29.91 A
Lima beans	−5	0.0	48.45 A	−7.93 AB	19.14 A
	−5	2.5	48.25 A	−7.25 AB	18.73 A
	−5	3.1	48.53 A	−6.99 A	18.72 A
	−20	0.0	48.93 A	−7.35 B	18.72 A
	−20	3.9	48.58 A	−7.07 AB	18.49 A
	−20	4.6	48.24 A	−6.83 AB	18.09 A
Peas	−5	0.0	32.64 A	−11.06 A	16.80 A
	−5	2.5	32.22 A	−10.79 A	16.53 A
	−5	3.1	32.12 A	−10.75 A	16.16 A
	−20	0.0	32.06 A	−10.90 A	16.24 A
	−20	3.9	31.78 A	−10.81 A	16.20 A
	−20	4.6	32.07 A	−10.55 A	16.30 A

[a] 0 = white, 100 = black.
[b] Negative a values indicate greenness; positive a values indicate redness.
[c] Negative b values indicate blueness; positive b values indicate yellowness.
[d] For a given product, dose-temperature combinations with the same letter are not significantly different ($P < 0.05$, analysis of variance, Tukey test).

D_{10}-value for this isolate of *L. monocytogenes* on surface-inoculated beef frankfurters is 0.49 kGy *(11)* when irradiation is carried out at 4°C. In these studies, D_{10} ranged from 0.505 to 0.613 kGy slightly below the freezing point (i.e., −5°C), depending on the vegetable substrate.

Complex, osmotically rich solutes suppress the freezing points of solutions; in a surface microniche, exudates or other compounds may have the effect of causing liquid water to persist at lower subfreezing temperatures, thereby increasing the mobility and efficacy of free radicals generated during irradiation. The net effect would be a more extensive bacterial kill than would otherwise be observed. Broccoli, the most anatomically complex vegetable examined, demonstrated the strongest sensitivity at −5°C, with a D_{10}-value significantly lower than that for any other vegetable. At ≤−10°C, broccoli's influence on D_{10} was similar to that of two of the three other vegetables. Surface anatomical complexity therefore seems not to play an important role in the mechanisms governing the effects seen. At decreasing temperatures, the significance of surface chemistry would be expected to decrease as surface solutions concentrate and ultimately freeze. Further research is required to more fully explain the different influence that each vegetable has on the suspended bacteria's D_{10}-value as temperature decreases.

When protocols involving ionizing radiation for the processing of frozen vegetables are designed, the antimicrobial efficacy of the process will be balanced against any negative changes to the product's sensory qualities. Doses that lead to extensive hydrolysis of pectins at refrigeration temperatures *(16)* cause less softening in the frozen state as a result of the reduced mobility of the free radicals *(15)*. However, the reduced mobility also reduces the antimicrobial efficacy, increasing the doses required. Doses sufficient to achieve a 5-$\log_{10}$ kill (3.9 and 4.6 kGy) caused significant softening in peas and broccoli when delivered at −20°C, but lower doses with comparable antimicrobial efficacy (2.5 and 3.1 kGy) caused no significant softening when delivered at −5°C. For peas and broccoli, the pattern of textural response to a specific radiation dose–temperature combination is clearly not the same as the sensitivity response pattern of *L. monocytogenes.* These results demonstrate that as a population of inoculated bacteria is reduced to a target level, the amount of associated radiation-induced softening may be controlled by adjusting the temperature of irradiation. It should be stressed, however, that the textural response was product-specific and that none of the four vegetables examined exhibited a loss of color associated with a specific radiation dose–temperature combination.

Chilled foods that incorporate frozen vegetables that have been thawed but not blanched may harbor *L. monocytogenes.* The results presented here demonstrate that ionizing radiation can effectively eliminate *L. monocytogenes* from frozen vegetables with little or no negative sensory impact, but these results also reinforce the difficulty in predicting the response of a particular frozen vegetable on the basis of data for any other frozen vegetable. To determine an optimal dose, these results should be considered in the context of industrial frozen vegetable processing and storage conditions. The irradiation response variables in these studies (i.e., color, texture, and antimicrobial efficacy) were influenced in complex ways by the suspending vegetable and the temperature of irradiation. An additional source of variation not addressed in the current work is the potential for isolate-based variation in radiation sensitivity and response to irradiation at decreasing frozen temperatures. While it has not been possible to develop a generalized predictive understanding of how these factors interact, additional research will elucidate this process and allow better design of irradiation protocols. As with other food-processing technologies, the applicability of irradiation (or lack thereof) to the processing of frozen vegetables will depend on the extent to which desirable outcomes (e.g., microbial control) are balanced against undesirable outcomes (e.g., loss of quality) under commercial processing conditions.

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